REMARKS/ARGUMENTS

Claims 9-38 are active. The claims have not been amended, but are represented for the convenience of the Examiner. The Applicants thank Examiner Kuhns for the courteous and helpful interview of September 14, 2005. Differences between chitosan gels and chitosan beads were discussed. The Applicants indicated that beads permitted yeast to desorb into the culture medium, while gels entrapped the yeast cells within a gel matrix. The Examiners queried why gels and beads would not be equivalents since the prior art gels probably had some yeast on their surfaces which might desorb. Ways to further distinguish the chitosan beads of the present invention from those of the prior art were discussed.

Election/Restriction

The Restriction Requirement has been made FINAL. Currently, the elected species is:

Carrier:	chitosan
Final product:	malt alcoholic beverage
Bioreactor:	fluidized bed.

The Applicants understand that upon an indication of allowability for a generic claim that additional species will be examined.

Rejection—35 U.S.C. 103

Claims 9, 10, 13-17, 21, 23, 25-28 and 33-37 were rejected under 35 U.S.C. 103(a) as being unpatentable over Sapporo Breweries, JP 6197749, in view of Szlavko, J. Amer. Soc. Brewing Chem. 2:59-60. The Applicants respectfully traverse this ground of rejection, since the prior art does not disclose or suggest (a) a process in which nonflocculent yeast are attached to chitosan beads, (b) does not provide a reasonable expectation of the benefits

obtained by a process using chitosan bead-immobilized nonflocculent yeasts, such as higher levels of floating yeast cells and lowered diacetyl (an undesirable "raw" flavor) content, and (c) does not contemplate a process employing a fluidized bed reactor in conjunction with chitosan-bead immobilized non-flocculent yeast.

Chitosan Beads v. Gels. The Applicants point out that the chitosan bead required by the present claims has the yeasts coated on their surfaces and that the surface-coated yeasts may readily desorb into the fermentation medium where they reduce diacetyl levels. On the other hand, the yeast in the prior art chitosan gels are double-fixed, that is embedded in the chitosan gel and then covered with a second layer of gel. Such embedded yeasts would not readily desorb into the fermentation medium and reduce diacetyl levels. Thus, the chitosan gel of the prior art is not the structural or functional equivalent of the chitosan beads of the invention.

The Official Action takes issue with the statement that "there is no or little yeast on the surface" of the chitosan gel and poses that this leaves open the possibility that some yeast is present on the gel surface. However, according to Sapporo (English abstract) the process is conducted with "immobilized yeast having the surface **covered** with a gel (emphasis added)". According to Sapporo [0008]:

> Although the yeast is fixed by the conventional method. . .it is required to **cover the front face with gel further** so that this fixed yeast may serve as aversion-conditions. By specifically covering the support by which yeast was fixed in the layer of gel, internal yeast can be aversion-and generation of diacetyls can be suppressed (emphasis added).

The prior art gel-immobilized yeasts are "double fixed". That is, fixed once in a gel and then fixed again by covering the conventionally fixed yeast with another layer of gel. Sapporo [[0016] exemplifies what happens if the yeasts are only conventionally fixed without covering them with the second layer of gel: single gel-fixed yeast produce high levels of

diacetyl and high levels (1.0×10^6) of "leaked yeast". On the other hand, in the Sapporo process using the double-fixed yeast "the leakage of yeast was not accepted at all". Thus, Sapporo teaches away from a process in which yeasts appear on the surface of a chitosan gel, since the Sapporo process uses double-fixed yeasts which are covered with a second gel layer. Moreover, the chitosan-gel based process described in comparative example in [0016] of Sapporo fails to produce the low diacetyl levels of the presently claimed process, the Sapporo method requires double fixation to achieve the lowered diacetyl levels [0001]. Furthermore, Sapporo fails to disclose or suggest a fluidized bed process employing non-flocculent yeasts fixed to a chitosan bead which reduces diacetyl levels.

On the other hand, the method of the present invention lowers diacetyl levels by using chitosan-bead immobilized, non-flocculent yeasts (see the bottom of page 3 of the specification). Chitosan beads comprise solidified chitosan in bead form and have a solidified chitosan layer on their surfaces. In the present invention, yeast is immobilized on the surface of the chitosan beads by soaking the beads in a yeast suspension (page 17, lines 7-10). Since in the process of the invention, the yeast is attached and immobilized on the surface of the chitosan beads, the yeast proliferates on the bead surface, is readily desorbable and floating yeast increases. As a consequence, the amount of diacetyl in the primary fermentation liquid is lowered and diacetyl is further efficiently reduced in any subsequent secondary fermentation (see the specification, page 9, line 10 to page 10, line 19). In addition, carbon dioxide produced as a result of fermentation is readily eliminated and fermentation delays do not occur since the nonflocculent yeast is immobilized on the surface of the chitosan beads. These aspects of the claimed process are simply not disclosed or suggested by Sapporo.

The Official Action previously indicated that "... it is clear that beads are in fact produced and used. On page 2, in the example, of the translation, the yeast are immobilized

in cells after the yeast and gelling material are expelled from double pipe". However, it is impossible to produce beads by the method described in Japanese Patent No. 6197749 and even were this process to produce beads, the yeast would be embedded in the beads and would not provide the beneficial effects of the beads of the present invention in which the yeast is immobilized on the surface.

Fluidized Bed Bioreactor and Durability of Chitosan Beads. The elected species subject to examination requires a fluidized bed bioreactor. Chitosan beads are suitable for use in such a bioreactor because they structurally durable and strong. However, the prior art chitosan gels are jelly-like and structurally weak and not suitable for such a process.

Use of a fluidized bed reactor with the more delicate double-fixed chitosan gels of Sapporo would likely produce the results shown in the comparative example [0016] of Sapporo, namely high diacetyl concentrations. Thus, one with ordinary skill in the art would not have selected chitosan gels for use in a fluidized bed reactor, since under such conditions the chitosan gel of the prior art would be broken down, would have released its yeast into the fermentation medium and would have expected to increase diacetyl levels as described in the Sapporo comparative example.

Non-flocculent yeast. The rejection posits that one with ordinary skill in the art would have had a reasonable expectation of success for raising tryptophol levels by immobilizing a non-flocculent yeast into the chitosan gels of Sapporo. As noted above, the yeasts of Sapporo are double-fixed and immobilized in a chitosan gel. Even if the Sapporo method employed non-flocculent yeasts, the yeasts would effectively be flocculated by virtue of being embedded in a gel. Thus, the Sapporo method inherently requires that the yeast (flocculent or non-flocculent) be in an immobilized flocculent-like form and yeast selection would not affect tryptophol levels since the non-flocculent yeast could not manifest its

functional properties, such as not sticking together, aggregating and decreasing yeast exposures to the fermentation medium.

Szlavko is cited as generally suggesting the non-flocculent yeast produces more tryptophol (a flavor component) than flocculent yeast. However, there is no suggestion in Szlavko that there is any difference at all in tryptophol levels produced by flocculent or non-flocculent yeasts when immobilized in a chitosan gel, or when used in a fluidized bed reactor. All that Szlavko discloses is that under certain process conditions, that certain non-flocculent yeasts produce more tryptophol than flocculent yeasts.

Moreover, Szlavko indicates that process conditions are critical for determining tryptophol levels. Szlavko indicates that both "top-cropping" and "non-flocculent" strains of *S. cerevisiae* formed higher levels of tryptophol than "bottom cropping" strains. However, under other process conditions, such as with the addition of glucose to the fermentation medium that bottom-cropping yeasts boosted tryptophol levels in "direct proportion" to the increase in glucose. Thus, Szlavko clearly shows that tryptophol production in *S. cerevisiae* is dependent on process conditions and not necessarily on the selection of a particular type of yeast.

Accordingly, Sapporo even in combination with Szlavko do not (a) disclose all the elements of the invention, namely non-flocculent yeasts immobilized on chitosan beads employed in a fluidized bed reactor, or non-flocculent yeasts meeting the criteria stated in Claim 9; (b) provide no motivation for substituting non-flocculent yeast for the immobilized yeasts used in the Sapporo process, and (c) do not provide a reasonable expectation of success for reducing diacetyl concentrations using the methods of the invention, namely fixing non-flocculent yeasts on chitosan beads in a fluidized bed reactor. On the other hand, the process of the present invention provides a superior product as shown in the experimental data of record.

Scope of improvements by using nonflocculent yeast. The Official Action indicates that the Applicants' evidence of superior results is irrelevant since there is some other motivation (i.e., increasing tryptophol content) for using non-flocculent yeasts. The Applicants disagree for the reasons set forth above. They also reiterate that the previously reported results are representative of the superior results obtained by the method of the invention. The important property of the representative yeast strains NA-3 and NA-4 is their lack of flocculation. This ability is shared by other non-flocculent yeasts. By employing a nonflocculent yeast "the yeast is fully prevented from flocculating within the bioreactor and thereby sedimenting and precipitating" (page 9, lines 12-15) and "the number of floating yeast cells upon the end of fermentation is stably maintained at a level higher than that in the case where a conventional flocculent yeast is used". The higher number of nonflocculent yeast cells results in a reduction of diacetyl (an undesirable "raw" flavor). A process using flocculent yeast would result in flocculation and precipitation of the yeast from the fermentation liquid, fewer floating yeast cells, and consequently a reduced amount of diacetyl (the undesirable "raw" flavor) removed during both primary and any secondary fermentation. Since nonflocculent yeasts would share these properties, i.e., they would not flocculate and precipitate, in general, any nonflocculent yeast would be expected to function as described within the invention and these properties would not be expected to be limited to only strains NA-3 and NA-4. Moreover, the claims themselves, see e.g., the last wherein clause in Claim 9, clearly describe the characteristics of a nonflocculent yeast.

Accordingly, the Applicants respectfully request that this rejection be withdrawn since the cited prior art does not teach all the elements of the invention including both chitosan beads and a process using a fluidized bed reactor. Second, even if the chitosan gel embedded yeasts are deemed to be equivalents of the chitosan bead-immobilized yeast of the invention, there is no motivation to substitute a non-flocculent yeast into the gels of Sapporo,

since such a substitution essentially creates a flocculent yeast by virtue of yeast cell double fixation in a gel. Third, the prior art does not provide a reasonable expectation that any benefit would arise from such a substitution since Szlavko indicates that process conditions are critical to determining tryptophol levels; and the cited prior art does not disclose or suggest the process conditions required by the present invention.

CONCLUSION

In view of the above amendments and remarks, the Applicants respectfully submit that this application is now in condition for allowance. Early notification to that effect is earnestly solicited.

Respectfully submitted,

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